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(54) Title: RECEPTOR/LIGAND BIOSENSOR

(57) Abstract

The present invention provides a biosensor for use in detecting the oligomerization of receptors. The biosensor comprises an electrode and a bilayer membrane having a top and a bottom layer. The bottom layer is proximal to and connected to the electrode in a manner such that a space exists between the membrane and the electrode. The membrane comprises a closely packed array of amphiphilic molecules and a plurality of ion channels comprising first half membrane spanning monomers dispersed in the top layer and second half membrane spanning monomers dispersed in the bottom layer. The first half membrane spanning monomers are capable of lateral diffusion within the upper layer while the second half membrane–spanning monomers are prevented from lateral diffusion within the bottom layer. Receptors are attached to an end of at least a proportion of the first half membrane–spanning monomers proximal to the surface of the membrane and the oligomerization of the receptors causes a change in the conductance or impedance of the membrane. The biosensors are useful in screening compounds for their ability to promote or interfere with receptor oligomerization.

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Receptor/Ligand Biosensor

The present invention relates generally to anchoring molecules to model membrane systems and to the use of anchored molecules in assays of inter molecular interactions.

Cell surface biomolecules, such as receptors, often need to be in an oligomerized or clustered form to enable signalling function and interactions with ligands. Such ligands include growth factors, cytokines, hormones, surface exposed components on cells, subcellular viral, subviral particle and other infectious agents (eg Science **275**, 1261-64, 1997). By virtue of their ability to undergo multimeric interactions, oligomerized receptors have the potential to interact stably with ligands of low affinity. Such oligomerization is usually essential for transmembrane signalling and receptor function. However, for many receptors the affinity of self-association or interaction with ligands is not high enough to allow detection using conventional binding techniques, which often require covalent labelling, solubilization with detergents, or immobilization of the receptor or ligand onto the solid sensing surface of existing biosensors. These methods are suited to the study of relatively high affinity interactions and they generally rely on the ability of the molecules to interact in solution or to maintain stable interaction after cell disruption. Since the effective receptor/ligand concentration in solution is significantly reduced compared to that on the two-dimensional surface of a cell (where molecules can oligomerize or cluster and interact stably with other molecules through multimeric interactions) these methods are limited in their ability to detect interactions of low affinity.

Since oligomerization of receptors is essential for their functional activity, assay methods which allow detection of this oligomerization process are highly suitable for screening of compounds that influence receptor aggregation and hence cellular functions triggered by that process. Such compounds would be candidates for drugs or other therapeutic agents relevant to disease states in which cellular transmembrane signalling events are involved. Oligomerization of receptors may be mediated by their extramembraneous or transmembrane domains.

Atomic force microscopy (also known as scanning probe microscopy) allows three-dimensional imaging and measurement of structures ranging in size from atomic dimensions to microns, and is revolutionary in its ability to resolve structures never seen before. The development of optical biosensors

has permitted the monitoring of the interaction between macromolecules in real time. To date, both of these techniques generally have been used with the receptor or the ligand molecule covalently attached to or immobilised onto a solid surface. Recently a technique has been described in which a linkage of a recombinant hexa-histidine-tagged protein with nitrilotriacetic acid (NTA) is used to reversibly immobilize a hexa-histidine-tagged protein onto the solid sensing surface of a BIAcore surface plasmon resonance biosensor. The formation of a hybrid alkanethiol/phospholipid membrane on the BIAcore sensing surface also has been described, enabling analysis of the binding of streptavidin to biotinylated phosphatidylethanolamine in the bilayer. These prior art techniques do not permit an analysis of receptor interactions under conditions that mimic a cell surface and that allows lateral mobility of the molecule, as well as the possibility for oligomerization and multimeric interactions.

The present inventors have developed an apparatus which can be used to detect the oligomerization of receptors, receptor/ligand interaction and to screen compounds for their ability to interfere or promote such oligomerization or interaction. The apparatus is best described as a biosensor in that it involves the use of lipid membranes. Membranes for the use in biosensors have been disclosed In international Patent Application Nos PCT/AU88/00273, PCT/AU89/00352 PCT/AU90/00025 and PCT/AU92/00132. The disclosure of these applications is included herein by reference.

As disclosed in these applications, suitably modified lipid molecules may be caused to assemble into an electrode/ionic reservoir/insulating bilayer combination that is suitable for incorporation of ion channels and ionophores. It is also disclosed that the conductance of these membranes is dependent on the presence or absence of an analyte. In bilayer membranes in which each layer includes ion channel monomers, the conductance of the membrane is dependent on the lining up of the monomers in each layer to form continuous ion channels which span the membrane. As these continuous ion channels are constantly being formed and destroyed, the conductance of the membrane is dependent on the lifetimes of these continuous ion channels.

In a first aspect, the present invention consists in a biosensor for use in detecting the oligomerization of receptors, the biosensor comprising an

electrode and a bilayer membrane having a top and a bottom layer, the bottom layer being proximal to and connected to the electrode in a manner such that a space exists between the membrane and the electrode, the membrane comprising a closely packed array of amphiphilic molecules and a plurality of ion channels comprising first half membrane spanning monomers dispersed in the top layer and second half membrane spanning monomers dispersed in the bottom layer, the first half membrane spanning monomers being capable of lateral diffusion within the upper layer and the second half membrane-spanning monomers being prevented from lateral diffusion within the bottom layer, receptors being attached to an end of at least a proportion of the first half membrane-spanning monomers proximal to the surface of the membrane, the oligomerization of the receptors causing a change in the conductance or impedance of the membrane.

In a preferred embodiment of the present invention the membrane includes membrane spanning amphiphiles which are prevented from lateral diffusion within the membrane.

In a second aspect, the present invention consists in a biosensor for use in detecting the oligomerization of receptors, the biosensor comprising an electrode and a bilayer membrane having a top and a bottom layer, the bottom layer being proximal to and connected to the electrode in a manner such that a space exists between the membrane and the electrode, the membrane comprising a closely packed array of amphiphilic molecules, membrane spanning amphiphiles which are prevented from lateral diffusion within the membrane and a plurality of ion channels comprising first half membrane spanning monomers dispersed in the top layer and second half membrane spanning monomers dispersed in the bottom layer, the first half membrane spanning monomers being capable of lateral diffusion within the upper layer and the second half membrane-spanning monomers being prevented from lateral diffusion within the bottom layer, receptors being attached to either an end of at least a proportion of the first half membrane-spanning monomers proximal the surface of the membrane and to an end of at least a proportion of the membrane-spanning amphiphiles proximal the surface of the membrane, the oligomerization of the receptors causing a change in the conductance or impedance of the membrane.

In a third aspect, the present invention consists in a biosensor for use in detecting receptor/ligand interaction, the biosensor comprising an

electrode and a bilayer membrane having a top and a bottom layer, the bottom layer being proximal to and connected to the electrode in a manner such that a space exists between the membrane and the electrode, the membrane comprising a closely packed array of amphiphilic molecules and a plurality of ion channels comprising first half membrane spanning monomers dispersed in the top layer and second half membrane spanning monomers dispersed in the bottom layer, the first half membrane spanning monomers being capable of lateral diffusion within the upper layer and the second half membrane-spanning monomers being prevented from lateral diffusion within the bottom layer, receptors being attached to an end of a proportion of the first half membrane-spanning monomers proximal the surface of the membrane and ligands being attached to the remainder, the interaction of the receptors with the ligands causing a change in the conductance or impedance of the membrane.

In a preferred embodiment of the present invention the membrane includes membrane spanning amphiphiles which are prevented from lateral diffusion within the membrane.

In a fourth aspect, the present invention consists in a biosensor for use in detecting receptor/ligand interaction, the biosensor comprising an electrode and a bilayer membrane having a top and a bottom layer, the bottom layer being proximal to and connected to the electrode in a manner such that a space exists between the membrane and the electrode, the membrane comprising a closely packed array of amphiphilic molecules, membrane spanning amphiphiles which are prevented from lateral diffusion within the membrane and a plurality of ion channels comprising first half membrane spanning monomers dispersed in the top layer and second half membrane spanning monomers dispersed in the bottom layer, the first half membrane spanning monomers being capable of lateral diffusion within the upper layer and the second half membrane-spanning monomers being prevented from lateral diffusion within the bottom layer, receptors being attached to either an end of at least a proportion of the first half membrane-spanning monomers proximal the surface of the membrane or to an end of at least a proportion of the membrane-spanning amphiphiles proximal the surface of the membrane, and ligands being attached to the other of at least a proportion of the first half membrane-spanning monomers proximal to the surface of the membrane or the end of the membrane-spanning amphiphiles

proximal to the surface of the membrane, receptor/ligand interaction causing a change in the conductance or impedance of the membrane.

In a fifth aspect the present invention consists in a method of screening a compound for the ability to interfere with receptor oligomerization or receptor/ligand interaction, the method comprising adding the compound to the biosensor of the first, second, third or fourth aspect of the present invention and detecting change in the impedance or conductance of the membrane.

In a preferred embodiment of the invention, the first and second half membrane spanning monomers are gramicidin or one of its derivatives.

In a further preferred embodiment of the present invention, the bilayer membrane is attached to the electrode via linking molecules such that a space exists between the membrane and the electrode. Preferred linking molecules are those disclosed in application PCT/AU92/00132. In yet a further preferred embodiment of the present invention, the second half membrane spanning monomers are attached to the electrode via linker groups.

In yet another preferred embodiment of the present invention, the bilayer membrane includes membrane spanning lipids, similar to those found in archaebacteria.

Due to ability of the first half membrane spanning monomers to diffuse laterally within the membrane the biosensors of the present invention are able to mimic the interactions of receptors as they occur at the cell surface. The receptors will typically be receptor domains, which may be composed of proteins, protein fragments, proteoglycans, glycoproteins, or oligosaccharides. The biosensors of the present invention may be also used to examine interactions between DNA and DNA binding proteins.

Many cellular responses are initiated through the oligomerisation of two or more large protein receptors. Receptors that fall into this category are the hematopoietic receptor family, epidermal growth factor receptor family, the insulin receptor family, the colony stimulating receptor family and the cytokine receptor family. The tethered membrane technology can be used to screen potential drugs for their ability to block protein-protein interactions, or to cause protein-protein interactions. Specific examples of receptors

which may be used in the present invention include CD2, CD4, CD48, CD59, CD94, B7.1, Epo receptors and Erb receptors.

The receptors and ligands may be attached to the membrane using a variety of strategies a number of which are exemplified in the patent applications referred to above. Other strategies include but are not limited to metal chelation of a suitably molecularly engineered or chemically attached terminal group of an expressed protein, glycoprotein, proteoglycan or oligosaccharide (eg the inclusion of a hexahistidine tag) to a functionalised moiety on a compound on the membrane surface (eg a metal chelating group such as NTA (nitrilo triacetic acid)). Another possibility is a specifically engineered receptor or related fragment containing a suitably located peptide strand which spontaneously inserts into the membrane.

In order that the nature of the present invention may be more clearly understood preferred forms thereof will now be described with reference to the following non-limiting examples.

EXAMPLES

Abbreviations

PBS	Phosphate buffered saline
EDC	1-(3-Dimethylamino)propyl-3-ethylcarbodiimide hydrochloride
NHS	N-hydroxy succinimide
NTA	Nitilotriacetic acid

The structure of "linker lipid A" is shown in Figure 1; the structure of "linker gramicidin B" is shown in Figure 2 in which gA is gramicidin, the structure of which is shown in Figure 3; the structure of MSLOH and MSL4XB are shown in Figure 4; the structure of "biotinylated gramicidin E" where n=5, is shown in Figure 5 in which gA is gramicidin, the structure of which is shown in Figure 3.

The cell surface receptor B7.1 (CD80) is a glycoprotein expressed predominantly on the surface of B-cells, dendritic cells, monocytes and peritoneal macrophages. The receptor is involved in the interaction between T cells and antigen-presenting cells, and plays an important role in T cell

activation and the induction of antigen-specific immune responses, by providing a costimulatory signal to the T cells.

The B7.1 receptor has a molecular mass of approximately 60 kDa; it possesses two extracellular immunoglobulin-like domains, a hydrophobic transmembrane region and a short cytoplasmic tail. The extracellular region of B7.1 contains binding sites for its cognate receptors CD28 and CTLA-4 on the surface of T cells. Upon recognition of antigen by the T cell antigen receptor (TCR), the binding of B7.1 to CD28 provides a co-stimulatory signal essential for T cell activation. The murine and human B7.1 molecules show considerable phylogenic conservation: human B7.1 can bind and signal through murine CD28 and CTLA-4, and vice versa.

The erbB family of growth factor receptors are cell surface receptors are commonly overexpressed in breast and ovarian carcinomas. The receptor for EGF, called ErbB-1, can undergo extensive heterodimerisation with three related membrane receptor proteins:- an orphan receptor called ErbB-2, and two receptors for the Neu differentiation factor ligand (NDF, heregulin, HRG) called ErbB-3 and ErbB-4. Although no known ligand binds directly to erbB-2, this receptor appears to be the preferred heterodimerising partner for the other erbB receptors. The rate of ligand dissociation for a heterodimer containing ErbB-2 is also thought to be slower than for the other dimers.

The experiments described in example 1 and 2 were performed using a recombinant form of murine B7-1. The recombinant B7.1 used consisted of the entire amino acid sequence of the extracellular region of murine B7.1 and a hexahistidine (6H) tag at the carboxyl terminal end of the protein (corresponding to the membrane-proximal end of the native B7.1 protein). The 6H tag was engineered to enable the receptor to be linked to nitrilotriacetic acid (NTA) groups through the formation of a metal-chelating linkage between the six successive histidine residues on the protein and the NTA. The recombinant protein was produced using standard molecular biology techniques in which the murine B7.1 gene was engineered to encode the extracellular region of B7.1 with a hexahistidine tag (B7.1-6H); the gene was then cloned and expressed in insect SF9 and High-5 cells using the baculovirus expression system. Recombinant B7.1-6H protein was expressed in the culture supernatants of High-5 cells transfected to express the B7.1-6H gene, and correct expression of the B7.16H protein was confirmed by

SDS-PAGE analysis, and by immunoblotting and immunoprecipitation with the commercially available murine B7.1-specific monoclonal antibody 16-10A1. For scale-up expression of the protein, supernatants of High-5 cells expressing the recombinant B7.1-6H protein were pooled and the recombinant B7.1 protein was purified by Ni-NTA chromatography followed by gel filtration on FPLC. As judged by SDS-PAGE analysis, the purity of the B7.1-6H produced by this method was in excess of 95%.

Example 1

A glass slide was evaporatively coated with a 200Å chromium adhesive layer, followed by a 1000Å layer of gold. The gold coated substrate was placed in a 50 ml ethanolic solution containing the components as listed in Table 1, in the concentrations shown.

Table 1

COMPONENT	MOLARITY
Linker Lipid A	370 μM
Mercaptoacetic acid Disulfide	185 μM
MSL4XB	27.75 nM
MSLOH	5.5 μM
Linker gramicidin B	55.5nM

The gold coated substrate was placed into this solution within five minutes of preparation. The gold coated substrate was left in this solution for 60 minutes, and then rinsed copiously with ethanol, and then immersed in ethanol for 2-3 hours. The gold slide was then rinsed with ethanol and assembled in an electrode holder such that an electrode is defined, that for the current examples has an area of approximately 11mm^2. Then, 10 μl of an ethanolic solution of 1,2-di(3RS,7R,11R-phytanyl)-glycero-3-phosphocholine and 1,2-di(3RS,7R,11R-phytanyl)glycerol in a 7:3 ratio, 3mM total lipid concentration, containing biotinylated gramicidin E where n=5, in a concentration such that the ratio of total lipid to gramicidin derivative is

67,000:1 was added to the surface of the gold electrode and then rinsed with three washes of 150 µl PBS, leaving 100 µl PBS above the electrode surface. A silver counter electrode was immersed in the PBS solution, and the counter electrode and the sensing electrode connected to an impedance bridge. A DC offset of -300mV was applied to the sensing electrode during AC measurement. Then 50 µl of 0.1 mg/ml solution of streptavidin bearing multiple NTA groups (prepared by treating streptavidin with EDC/NHS, followed by lysine-NTA) was added to the electrode well and left for three to five minutes, A 5 µl solution of 0.1 mg/ml biotinylated NTA (prepared by reacting lysine-NTA with biotin-NHS) was added and the solution left for a further 5 minutes, prior to rinsing with PBS (3 x 100 µl). 15 µl of a 0.33 mg/ml solution of the protein B7, bearing a 6-His tag, was then added and after 15 minutes, a monoclonal antibody to the B7 protein (5 µl of a 0.7 mg/ml solution) was added. A gating of 8.1% was observed in the impedance spectrum. A control experiment using native streptavidin and omitting the addition of biotinylated NTA, showed a minimal gating of 2.5%.

Lysine NTA was prepared following the route of Schmidt *et al.*, *J.Am.Chem.Soc.*, 1994, 116, 8485.

Example 2

A tethered bilayer membrane was prepared as described in Example 1.

The 5 µl of 0.1 mg/ml solution of streptavidin bearing multiple NTA groups was added to the electrode well and left for three to five minutes. A 5 µl solution of 0.1 mg/ml biotinylated NTA (prepared by reacting lysine-NTA with biotin-NHS) was added and the solution left for a further 5 minutes, prior to rinsing with PBS (3 x 100 µl). 15 µl of a 0.33 mg/ml solution of polybiotinylated protein B7 bearing a 6-His tag, (prepared by reacting the 6-His tagged protein with biotin-NHS) was then added and after 20 minutes, 5 µl of 0.1 mg/ml solution of streptavidin was added to the electrode well. A gating of 10.8% was then observed in the impedance spectrum. A control experiment using native streptavidin and omitting the addition of biotinylated NTA, showed a modest gating of 4.1%.

Example 3

A tethered bilayer membrane was prepared as described in Example 1. A solution of streptavidin (5µl of 0.1 mg/ml solution) was added to the electrode well and left for three to five minutes and then rinsed with PBS (3 x 100µl). A solution of biotinylated anti-FLAG Fab' (5µl, 2µM) was then added, and after five minutes the well was rinsed with PBS (3 x 100µl). A solution of FLAG-labelled HRG (0.2µl of 0.3µM solution, well concentration 6nM) was added and the gating of 15% observed by impedance spectroscopy.

Example 4

A tethered bilayer membrane was prepared as described in Example 1. A solution of streptavidin (5µl of 0.1 mg/ml solution) was added to the electrode well and left for three to five minutes and then rinsed with PBS (3 x 100µl). A solution of biotinylated anti-FLAG Fab' (5µl, 2µM) was then added, and after five minutes the well was rinsed with PBS (3 x 100µl). A 1:1 mixture of FLAG-labelled erbB2 and erbB4 receptors was added to the well (2µl of a 4µM solution, well concentration 80nM) and after 10 minutes the well was rinsed with PBS (3 x 100µl). No gating was observed by impedance spectroscopy at this stage. A solution of FLAG-labelled HRG (0.2µl of 0.3µM solution, well concentration 6nM) was added and the gating of ~15% was observed by impedance spectroscopy.

As will be readily appreciated by those skilled in the art the present invention provides a method of assaying interactions between membrane anchored molecules and between anchored molecules and molecules capable of interacting therewith. More particularly, the present invention is useful to study interactions between receptors and between a receptor and a ligand by anchoring the extra membranous or transmembrane region of the receptor biomolecule on to a fluid membrane system.

The present invention thus provides for the anchoring of receptor molecules onto ion channel containing supported bilayers, that enable the molecules to diffuse laterally and interact. This technology is ideal in a preferred embodiment for studying receptor-receptor and receptor-ligand

interactions in a membrane system using ion channels with electrical impedance detection.

It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

CLAIMS:-

1. A biosensor for use in detecting the oligomerization of receptors, the biosensor comprising an electrode and a bilayer membrane having a top and a bottom layer, the bottom layer being proximal to and connected to the electrode in a manner such that a space exists between the membrane and the electrode, the membrane comprising a closely packed array of amphiphilic molecules and a plurality of ion channels comprising first half membrane spanning monomers dispersed in the top layer and second half membrane spanning monomers dispersed in the bottom layer, the first half membrane spanning monomers being capable of lateral diffusion within the upper layer and the second half membrane-spanning monomers being prevented from lateral diffusion within the bottom layer, receptors being attached to an end of at least a proportion of the first half membrane-spanning monomers proximal to the surface of the membrane, the oligomerization of the receptors causing a change in the conductance or impedance of the membrane.

2. A biosensor as claimed in claim 1 in which the membrane includes membrane spanning amphiphiles which are prevented from lateral diffusion within the membrane.

3. A biosensor for use in detecting the oligomerization of receptors, the biosensor comprising an electrode and a bilayer membrane having a top and a bottom layer, the bottom layer being proximal to and connected to the electrode in a manner such that a space exists between the membrane and the electrode, the membrane comprising a closely packed array of amphiphilic molecules, membrane spanning amphiphiles which are prevented from lateral diffusion within the membrane and a plurality of ion channels comprising first half membrane spanning monomers dispersed in the top layer and second half membrane spanning monomers dispersed in the bottom layer, the first half membrane spanning monomers being capable of lateral diffusion within the upper layer and the second half membrane-spanning monomers being prevented from lateral diffusion within the bottom layer, receptors being attached to either an end of at least a proportion of the first half membrane-spanning monomers proximal the surface of the membrane and to an end of at least a proportion of the membrane-spanning amphiphiles proximal the surface of the membrane, the oligomerization of

the receptors causing a change in the conductance or impedance of the membrane.

4. A biosensor for use in detecting receptor/ligand interaction, the biosensor comprising an electrode and a bilayer membrane having a top and a bottom layer, the bottom layer being proximal to and connected to the electrode in a manner such that a space exists between the membrane and the electrode, the membrane comprising a closely packed array of amphiphilic molecules and a plurality of ion channels comprising first half membrane spanning monomers dispersed in the top layer and second half membrane spanning monomers dispersed in the bottom layer, the first half membrane spanning monomers being capable of lateral diffusion within the upper layer and the second half membrane-spanning monomers being prevented from lateral diffusion within the bottom layer, receptors being attached to an end of a proportion of the first half membrane-spanning monomers proximal the surface of the membrane and ligands being attached to the remainder, the interaction of the receptors with the ligands causing a change in the conductance or impedance of the membrane.

5. A biosensor as claimed in claim 3 or claim 4 in which the membrane includes membrane spanning amphiphiles which are prevented from lateral diffusion within the membrane.

6. A biosensor for use in detecting receptor/ligand interaction, the biosensor comprising an electrode and a bilayer membrane having a top and a bottom layer, the bottom layer being proximal to and connected to the electrode in a manner such that a space exists between the membrane and the electrode, the membrane comprising a closely packed array of amphiphilic molecules, membrane spanning amphiphiles which are prevented from lateral diffusion within the membrane and a plurality of ion channels comprising first half membrane spanning monomers dispersed in the top layer and second half membrane spanning monomers dispersed in the bottom layer, the first half membrane spanning monomers being capable of lateral diffusion within the upper layer and the second half membrane-spanning monomers being prevented from lateral diffusion within the bottom layer, receptors being attached to either an end of at least a proportion of the first half membrane-spanning monomers proximal the surface of the membrane or to an end of at least a proportion of the membrane-spanning amphiphiles proximal the surface of the membrane, and ligands being

attached to the other of at least a proportion of the first half membrane-spanning monomers proximal to the surface of the membrane or the end of the membrane-spanning amphiphiles proximal to the surface of the membrane, receptor/ligand interaction causing a change in the conductance or impedance of the membrane.

7. A biosensor as claimed in any one of claims 1 to 6 in which the first and second half membrane spanning monomers are gramicidin or one of its derivatives.

8. A biosensor as claimed in one of claims 1 to 7 in which the bilayer membrane is attached to the electrode via linking molecules such that a space exists between the membrane and the electrode.

9. A biosensor as claimed in one of claims 1 to 8 in which the second half membrane spanning monomers are attached to the electrode via linker groups.

10. A method of screening a compound for the ability to interfere with receptor oligomerization or receptor/ligand interaction, the method comprising adding the compound to the biosensor as claimed in any one of claims 1 to 9 and detecting change in the impedance or conductance of the membrane.

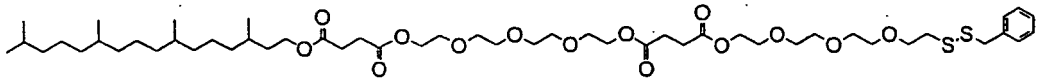

Figure 1

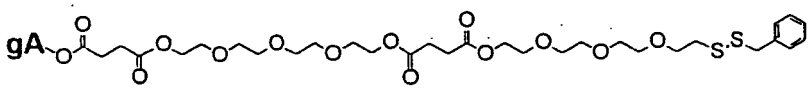

Figure 2

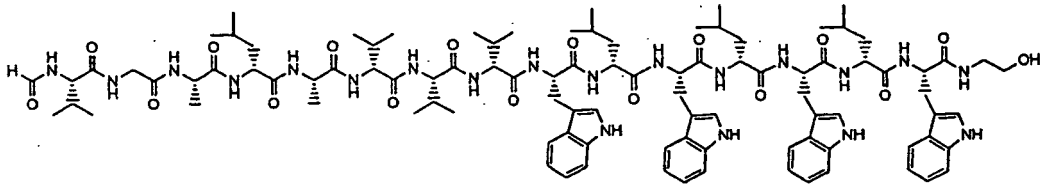

Figure 3

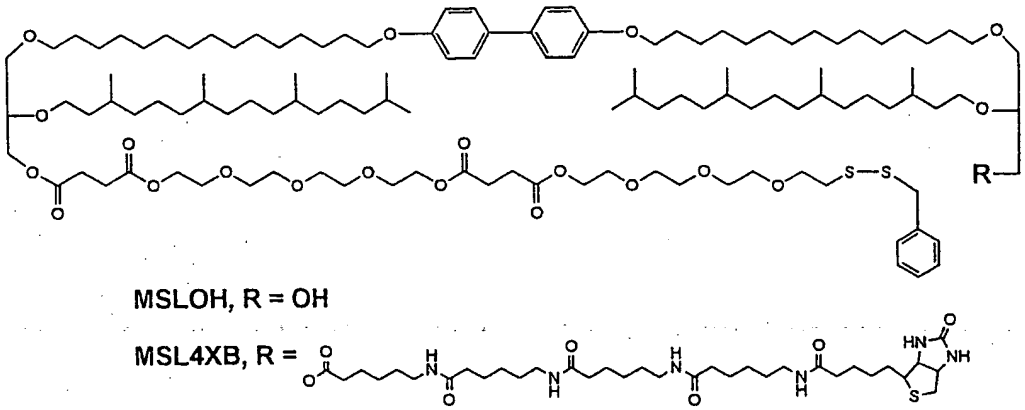

Figure 4

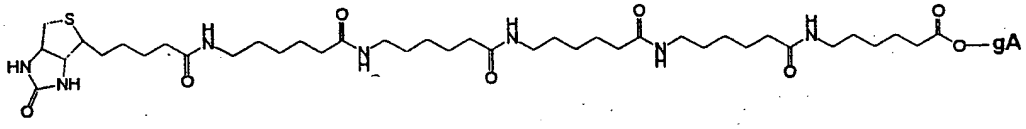

Figure 6

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00417

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: G01N 27/327, 27/333

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC G01N 27/327, 27/333

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT membran: , recept:
Chem. Abs. (biosensors OR bioelectrodes) AND (membranes) AND (ionophore OR ionophores)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AU 38643/95 A (Australian Membrane & Biotechnology Research Institute) 6 June 1996	1-10
Y	AU 59925/96 (692107) (Australian Membrane & Biotechnology Research Institute) 22 January 1997	1-10
Y	AU 65327/94 A (Australian Membrane & Biotechnology Research Institute) 8 November 1994	1-10

[X] Further documents are listed in the continuation of Box C

[X] See patent family annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search
5 August 1998

Date of mailing of the international search report
7 AUG 1998

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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 98/00417

C (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	AU 56188/94 (663243) (Australian Membrane & Biotechnology Research Institute) 22 June 1994	1-10
Y	AU 50334/90 (623747) (Australian Membrane & Biotechnology Research Institute) 24 August 1990	1-10
Y	AU 21279/88 (617687) (Commonwealth Scientific & Industrial Research Organisation) 1 March 1989	1-10
A	Science, vol. 275, 28 February 1997, pp 1261-1264, J. COHEN. -see Figure on page 1261	1-10

INTERNATIONAL SEARCH REPORT
Information on patent family members

International Application No.
PCT/AU 98/00417

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
AU	38643/95	WO	9615454	EP	791176	AU	9500/94
AU	59925/96	WO	9701091	EP	838027	AU	3668/95
AU	65327/94	WO	9424562	EP	695425	JP	8509807
		CA	2161084				
AU	56188/94	WO	9412875	EP	672251	CA	2150915
		JP	8504943	US	5591647		
AU	50334/90	WO	9008783	EP	455705	EP	770874
		ES	2102364	US	5443955	JP	4504714
		DE	69030811	AT	153673	CA	2045640
AU	21279/88	WO	8901159	EP	382736	JP	3503209
		JP	2682859	US	5436170	US	5693477
		US	5741712	US	5766960	AT	113724
		CA	1335879	DE	3852036		

END OF ANNEX